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Resolving the confused identity of *Frankliniella panamensis* (Thysanoptera: Thripidae)

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Abstract

Morphological and molecular characters are provided for distinguishing two similar species of *Frankliniella* that are commonly found by quarantine authorities in international shipments of horticultural produce, particularly from Colombia where *panamensis* and *occidentalis* co-exist in greenhouses.

Key words: hind coxal microtrichia, CO1 gene, quarantine interceptions

Introduction

The genus *Frankliniella* currently comprises 234 described species worldwide (ThripsWiki 2017), of which almost 90% are from the Neotropics. Moreover, judging from almost 1000 unidentified slide-mounted *Frankliniella* specimens in the collections of the Natural History Museum, London, the species diversity in the Andean mountain chain has yet to be explored. Amongst those specimens it has not proved possible even to securely associate males and females to putative species, such is the variation in colour and structure within and among samples. Species recognition in this genus is notoriously difficult and, in providing a key to almost 80 species from Central America and the Caribbean, Mound and Marullo (1996) emphasized that some of their distinguishing couplets were particularly weak. This applies particularly to some of the small, yellow species, a situation that was further emphasized by Cavalleri and Mound (2012) in providing a key to about 40 *Frankliniella* species from Brazil. Taxonomic decisions become significant when the species involved are considered to be pests. For example, two small yellow *Frankliniella* species described from southern Brazil, *rodeos* and *zucchini,* cannot at present be distinguished morphologically from *gemina* with any certainty, despite the identification key by Cavalleri and Mound (2012). One of these three, *zucchini*, is considered a vector of the tospovirus *Zucchini lethal chlorosis virus* (Nakahara & Monteiro 1999). Moreover, there is a suggestion that, under experimental conditions, *gemina* may be a vector of the tospoviruses *Tomato spotted wilt virus* and *Groundnut ringspot virus* (Borbon *et al*. 1999). Determination as to whether one or three species are involved in this complex requires studies of biological and molecular characteristics among populations in southern Brazil and Argentina, because these species are not known from anywhere else. A rather different situation is the target of this contribution, in which a pair of difficult to distinguish species from the West coast of the Americas are commonly found in shipments of plant material around the world.

The Western Flower Thrips, *F. occidentalis*, although originally from Western America is a major pest worldwide (Kirk & Terry 2003). It is a variable species, in colour as well as body structure and chaetotaxy, and molecular variation has also been reported between populations (Rugman-Jones *et al*. 2010). Recognition of this

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species is a high priority for quarantine entomologists, and the most closely similar species within *Frankliniella* seem to be *crotalariae, intonsa, insularis* and *panamensis.* The first of these is a pure yellow species that is probably host specific to the flowers of *Crotalaria* species, and although it is introduced to Hawaii (Mound *et al*. 2016) it has not been reported in the world trade in horticultural products. The second species, *intonsa*, is widespread across the Northern hemisphere, and is commonly taken in quarantine. This species differs from the other three in having the postocular setae much shorter and the metanotum without a pair of campaniform sensilla. The third species, *insularis*, is widespread in South America and southern parts of the US. It is also introduced to Hawaii, and as it has been seen (by LAM) from both Singapore and Fiji, it is likely to be intercepted in quarantine. It is similar to *occidentalis* and *panamensis* in having a pair of metanotal campaniform sensilla and a moderately long posteromarginal comb on tergite VIII. The main problem in this group of four species is in distinguishing *panamensis* from *occidentalis*. Even the original description by Hood (1925) indicated that these two species were similar, and *panamensis* has sometimes been regarded as possibly a particularly dark strain of Western Flower Thrips. The situation has become important because this dark species is increasingly being found in shipments of flowers from Colombia by quarantine authorities in several countries. This contribution is intended to provide the means of unequivocally distinguishing these two species from each other, using both morphological and molecular methods.

Material and methods

Morphological studies were based on slide-mounted thrips in the collections that are maintained by each of the listed authors at their respective research institutes. We examined between us a total of 68 species of *Frankliniella* (Table 1), with most species being from collections in Miami, Washington and Canberra. For some species, long series of specimens were examined from locations throughout the Americas and the Caribbean, whereas some species were represented by few specimens. The species studied included representatives from all seven of the morphological groupings suggested by Moulton (1948).

TABLE 1. Distribution of microtrichia on hind coxae among *Frankliniella* species.

i. Hind-coxal microtrichia present (but particularly minute*)
achaeta; *auripes*; *australis*; *bertelsi**; *bruneri*; *brunnea**; *caudiseta*; *citripes*; *colombiana*; *crawfordi**; *crotalariae**; *curiosa*; *desantisi**; *deserticola**; *desmodii*; *distinguenda*; *diversa*; *ewarti*; *fallaciosa*; *floydandrei*; *frumenti*; *fulvipennis*; *gemina*; *graminis*; *hemerocallis*; *insularis*; *intonsa*; *invasor*; *kelliae*; *lantanae*; *minuta*; *musaeperda*; *oxyura*; *pallida*; *panamensis*; *parvula*; *pontederiae**; *pulchella**; *regia*; *schultzei*; *serrata*; *standleyana*; *trisetosa*; *tuttlei**; *tympanona**; *valdiviana*; *vargasi*; *varipes*; *xanthaner.*
ii. Hind-coxal microtrichia absent (or not detected)
aztecus; *bispinosa*; *borinquen*; *cephalica*; *curta*; *davidsoni*; *fusca*; *jamaicensis*; *konoi*; *longipennis*; *magellanica*; *melanommata*; *nakaharai*; *occidentalis*; *pestinae*; *platensis*; *tenuicornis*; *tritici*; *tuberosi.*

Molecular studies involved extracting total DNA using the DNeasy for Blood and Tissue kit (Qiagen, Valencia, CA, USA) as per the manufacturer's instructions. Specimens of *occidentalis* came from Colombia, India, Netherlands and Zambia, but *panamensis* specimens were all from Colombia (Table 2). Identification of these adults was carried out using the morphological character states discussed below. Adult specimens were used for non-destructive DNA extraction by incubation overnight in ATL buffer plus Proteinase K. DNA of the immature stages was extracted by physical disruption using micro-pestles. The final DNA eluted in 100 µL of AE buffer. For DNA barcoding of the COI gene region from the thrips samples, LCO1490 and HCO2198 primers (Folmer *et al*. 1994) or mtD-7.2F and mtD-9.2R (Brunner *et al.* 2002) were used. For all the PCR reactions and sequencing were conducted as per Li *et al.* (2015). The obtained DNA sequences were edited in Geneious Pro 10.0.6 (http://www.geneious.com, Kearse *et al.* 2012) and BLAST searched against the GenBank (Altschul *et al.* 1990) and/or BOLD databases (Ratnasingham & Herbert 2007). Multiple sequence alignment was performed using the Geneious aligner and Clustal W in Geneious. All the available sequences from *insularis, intonsa, occidentalis* and *panamensis* from this study and GenBank/BOLD were aligned and the representative haplotypes from each species were selected for the phylogenetic analysis. Phylogenetic trees were constructed using Bayesian (MrBayes)

method in Geneious under the default settings (Huelsenbeck & Ronquist 2001). Multiple runs were performed using the model GTR and rate variation gamma. The resulting perimeter files were inspected for chain convergence and mixing in Tracer 1.4 (Rambaut & Drummond 2007). The trees were rooted using *Thrips palmi* COI sequences as outgroup.

TABLE 2. Samples used for DNA studies.

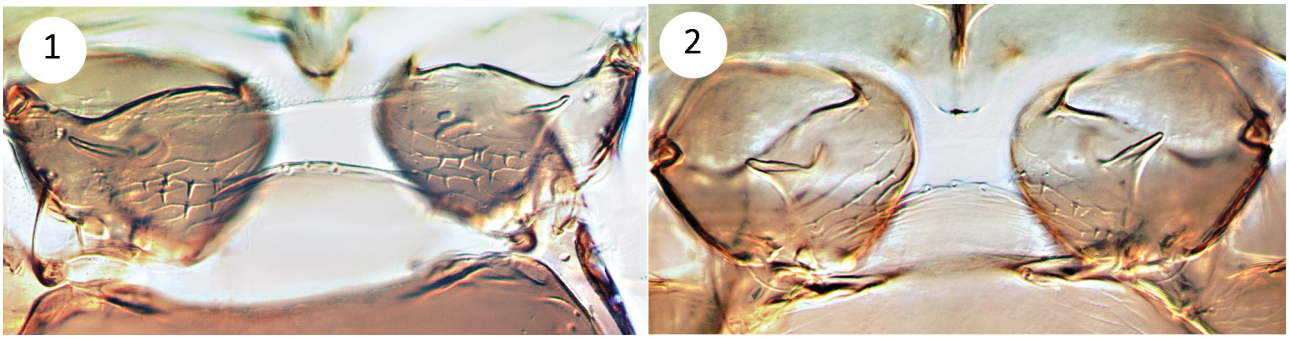
Species	Numbers of individuals	Country	Year	Host	BOLD acc#	Life stage
F. occidentalis	1	Colombia	2014	*Alstroemeria* sp.	BTQ001-17	larva
	6	Colombia	2014	*Aster* sp.	BTQ002-17 to BTQ007-17	larva
	2	Colombia	2015	*Rosa* sp.	BTQ008-17 BTQ011-17	larva
	2	Colombia	2014	*Rosa* sp.	BTQ009-17 BTQ010-17	adult
	8	Colombia	2014–2016	*Solidago* sp.	BTQ012-17 to BTQ019-17	larva
	1	India	2016	*Dianthus* sp.	BTQ020-17	larva
	1	India	2016	*Rosa* sp.	BTQ021-17	larva
	2	Netherlands	2015	*Eryngium* sp.	BTQ022-17 BTQ023-17	larva
	1	Netherlands	2014	*Hypercium* sp.	BTQ024-17	larva
	3	Netherlands	2015	*Limonium sinuatum*	BTQ025-17 to BTQ027-17	larva
	1	Zambia	2014	Snowpeas	BTQ028-17	larva
F. panamensis	2	Colombia	2014	*Alstroemeria* sp.	BTQ029-17 BTQ031-17	adult
	4	Colombia	2015	*Alstroemeria* sp.	BTQ030-17 BTQ032-17 to BTQ034-17	larva
	1	Colombia	2014	*Aster* sp.	BTQ035-17	adult
	1	Colombia	2016	cutflowers	BTQ036-17	larva
	3	Colombia*	2014	*Passiflora edulis*	BTQ037-17 to BTQ039-17	adult
	5	Colombia	2014–2015	*Rosa* sp.	BTQ040-17 BTQ041-17 BTQ043-17 BTQ045-17 BTQ046-17	larva
	5	Colombia	2014–2015	*Rosa* sp.	BTQ042-17 BTQ044-17 BTQ047-17 BTQ049-17	adult
	1	Colombia*	2014	*Rosmarinus officinalis*	BTQ050-17	adult
	2	Colombia*	2014	*Rubus glaucus*	BTQ051-17 BTQ052-17	adult
	1	Colombia	2015	*Solidago* sp.	BTQ053-17	larva
F. insularis	3	Colombia*	2014	*Citrus* sp.	BTQ054-17 to BTQ056-17	adult

Note: * Specimens obtained from overseas. All the other specimens were intercepted at New Zealand border.

Results and discussion

Morphological differences. Distinguishing slide-mounted specimens of *panamensis* from *occidentalis* has been based on the following character states: body colour usually darker; antennal segment V almost fully dark; ocellar setae pair III rather closer together; posteromarginal comb on tergite VIII longer and finer. However each of these is subjective, and recognition of isolated individuals is often not possible (Mound & Marullo 1996). In contrast, one previously unexplored character state has now been found to be consistently different between these two species. In *panamensis* the upper surface of the hind coxae bears a small and variable group of microtrichia (Figs 1, 2), whereas similar microtrichia have not been found on the hind coxae of any specimens of *occidentalis*. Although difficult to observe unless the specimens are reasonably well slide-mounted, this structural difference has the advantage that it applies to both sexes. This is important because the males of *panamensis* are yellow in colour, and thus not immediately connected with the dark females during routine identifications.

The presence of these coxal microtrichia is not, however, a character state that is unique to *panamensis* among *Frankliniella* species, because these microtrichia were detected on 49 of the 68 species studied (Table 1). Moreover, they are present in *crotalariae*, *insularis, intonsa* and *panamensis*, the four species that are considered closely related to *occidentalis*. Thus at present it seems that presence or absence of coxal microtrichia provides limited signal of affinity amongst these species. The number of coxal microtrichia, also their size and position on the upper surface of the coxae, varies between species. In *panamensis* there are about 10 stout microtrichia on the lines of sculpture, whereas in *crotalariae* there are only about 5 and these are much smaller. In *brunnea, pulchella*, and *pontederiae* the microtrichia are particularly minute. Establishment of "absence of microtrichia" for a species is clearly more difficult than establishing "presence". However, where long series were available, no intraspecific variation was found in the presence or absence of the coxal microtrichia among the species examined, with the sole exception of specimens currently identified as *brevicaulis* Hood.



FIGURES 1, 2. Microtrichia on upper surface of hind-coxae in *Frankliniella* species. **(1)** *panamensis*; **(2)** *intonsa.*

Molecular differences. Studies on the phylogenetic relationships between thrips have involved several genes (Buckman *et al*. 2013; Crespi *et al*. 1996), but for routine species identification the mitochondrial cytochrome oxidase subunit I (COI) gene has been employed as a marker (Glover *et al.* 2013; Kadirve *et al*. 2009). This technique is clearly useful for identifying immature stages, and is also claimed to have advantages where technical expertise to assess morphological variation of adult thrips is not available. Therefore, this study analyzed the COI sequence diversity from specimens of *occidentalis* and *panamensis* intercepted at the New Zealand border together with specimens obtained from overseas. The overseas *panamensis* specimens originated from the Colombia States of Antioquia and Norte De Santander, Bogota Plateau. The COI DNA barcoding data generated were used to identify all intercepted larvae and to investigate the intra- and inter- species variation for each species and the relationship between *occidentalis* and *panamensis*. The COI gene sequence was obtained from the following individuals: *occidentalis* (28), *panamensis* (25), *insularis* (3). All the COI sequences obtained (see BOLD accession numbers, Table 2) are high AT-rich, with an average of 68.7% for *occidentalis*, 71.2% for *panamensis* and 71.9% for *insularis*. The COI sequence comparison showed that the intra-species divergent is less than 2% for both *occidentalis* and *panamensis* sequenced in this study. In comparison, there is around 10–13% difference for COI sequences between *occidentalis* and *panamensis*. For *insularis* the differences from *occidentalis* and *panamensis* are around 15–18% and 13–16%, respectively. For *intonsa* the differences from *occidentalis* and *panamensis* are around 17–21% and 17–18%, respectively. Phylogenetic analysis did not reveal obvious separation

for specimens from different hosts and countries (Fig. 3). The analysis supported that *panamensis* and *occidentalis* are clearly separated into two clades, but the two species are closely related with 100% *pp* value support (Fig. 3). All the *occidentalis* sequences are formed well in one clade with 100% *pp* value support, but separated into two distinctive sub-clades (Fig. 3). One of these included the Lupin strain from *Lupinis arboreus* and *Lupinus polyphyllus* from New Zealand with strong support of 100% *pp* values; the other was the widespread pest or Glasshouse strain with weaker support of 61% *pp* values (Rugman-Jones *et al*. 2010 and Fig. 3).

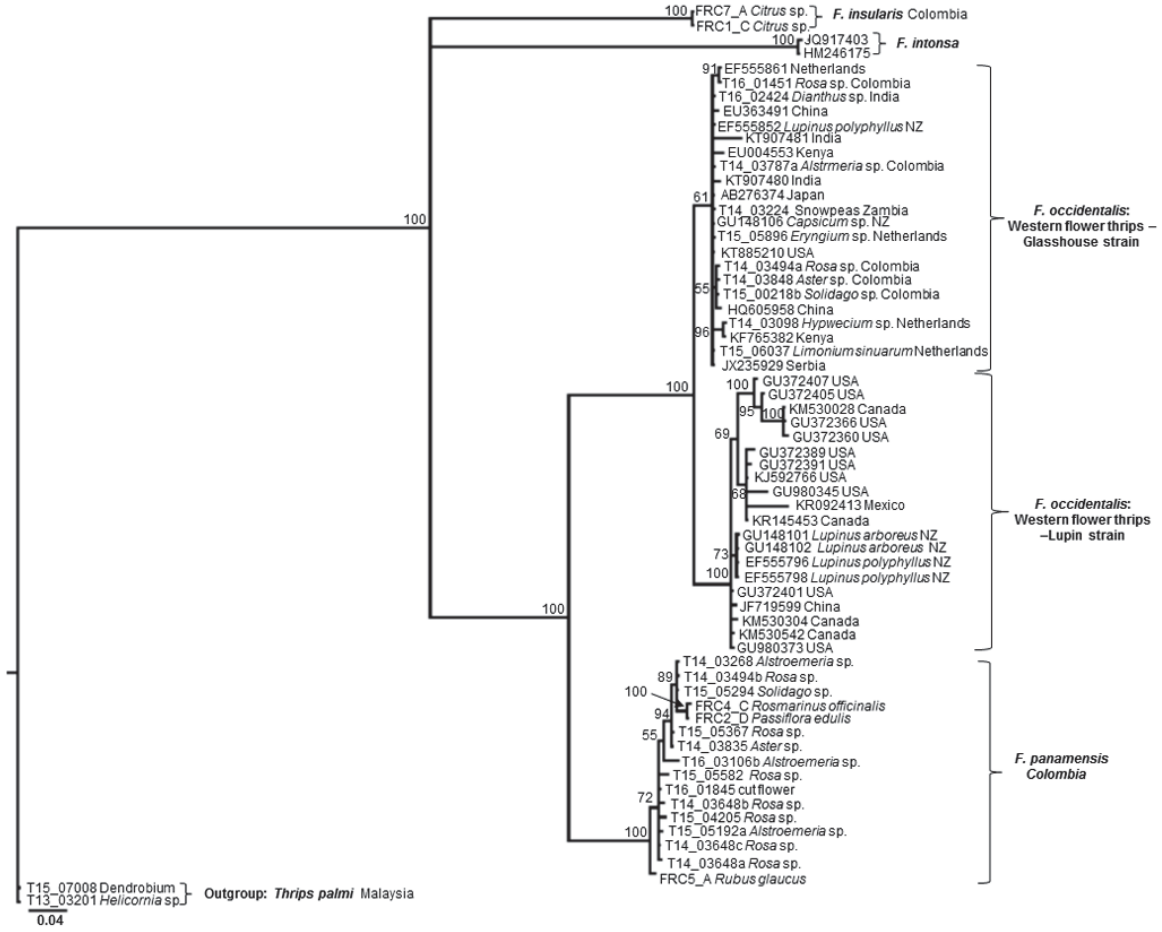


FIGURE 3. Bayesian phylogenetic tree inferred from sequences of the COI gene. Posterior probabilities greater than 50% are given on appropriate clades. Species name and GenBank Accession numbers are listed for each taxon. Host and countries for each taxon are listed if known.

Conclusion

We conclude that *panamensis* is a distinct species that can be readily distinguished from *occidentalis* by both morphological and molecular methods. However, a few comments are important on this conclusion and on the analysis presented here (Fig. 3). For economic entomology it is essential to remember that, as yet, there has been no demonstration of any biological differences between these two species. Moreover, further studies are required on the complex species that is called the Western Flower Thrips. There is clearly molecular complexity within *occidentalis*, with two distinct sub-clades evident, and interesting diversity within the clade referred to here as the Lupin strain. Finally, the data presented here do not confirm that *panamensis* and *occidentalis* are sister-species (Fig. 3); such a conclusion, together with any consideration of their relationship to *intonsa* or any other *Frankliniella* species will require investigations using several other genes.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403-410.
https://doi.org/10.1016/S0022-2836(05)80360-2

de Borbón, C.M., Gracia, O. & de Santis L. (1999) Survey of Thysanoptera occurring on vegetable crops as potential Tospovirus vectors in Mendoza, Argentina. *Revista de la Sociedad Entomológica Argentina*, 58 (3-4), 59–66.

Brunner, P.C., Fleming, C. & Frey, J.E. (2002) A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP-based approach. *Agricultural and Forest Entomology*, 4, 127–136.
https://doi.org/10.1046/j.1461-9563.2002.00132.x

Buckman, R.S., Mound, L.A., Whiting, M.F, (2013) Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. *Systematic Entomology*, 38, 123–133.
https://doi.org/10.1111/j.1365-3113.2012.00650.x

Cavalleri, A. & Mound, L.A. (2012) Toward the identification of *Frankliniella* species in Brazil (Thysanoptera: Thripidae). *Zootaxa*, 3270, 1–30.

Crespi, B.J., Carmean, D., Vawter, L. & Von Dohlen, C. (1996) Molecular phylogenetics of Thysanoptera. *Systematic Entomology*, 21, 79–87.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Glover, R.H., Collins, D.W., Walsh, K., & Boonham, N. (2010) Assessment of loci for DNA barcoding in the genus Thrips (Thysanoptera: Thripidae). *Molecular Ecology Resources*, 10, 51–59. [xPMID: 21564990]
https://doi.org/10.1111/j.1755-0998. 2009. 02723

Hood, J.D. (1925) New species of Frankliniella (Thysanoptera). *Bulletin of the Brooklyn Entomological Society*, 20, 71–83.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
https://doi.org/10.1093/bioinformatics/17.8.754

Kadirve, L.P., Srinivasan, R., Hsu, Y.C., Su, F.C. & DeLaPena, R. (2013) Application of cytochrome oxidase I sequences for phylogenetic analysis and identification of thrips species occurring on vegetable crops. *Journal of Economic Entomology*, 106, 408–418. [PMID:23448058]

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S. & Duran, C. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199

Kirk, W.D.J. & Terry, I. (2003) The spread of Western Flower Thrips *Frankliniella occidentalis* Pergande. *Agricultural and Forest Entomology*, 5, 301–310.
https://doi.org/10.1046/j.1461-9563.2003.00192.x

Li, D., Fan, Q.H., Waite, D.W., Gunawardana, D., George, S. & Kumarasinghe, L. (2015) Development and Validation of a Real-Time PCR Assay for Rapid Detection of Two-Spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae). *PLoS One*, 10, e0131887.
https://doi.org/10.1371/journal.pone.0131887

Moulton, D. (1948) The genus *Frankliniella* Karny, with keys for the determination of species (Thysanoptera). *Revista de Entomologia*, 19, 55–114.

Mound, L.A. & Marullo, R. (1996) The Thrips of Central and South America: An Introduction. *Memoirs on Entomology, International*, 6, 1–488.

Mound, L.A, Nakahara, S. & Tsuda, D.M. (2016) Thysanoptera-Terebrantia of the Hawaiian Islands: an identification manual. *ZooKeys*, 549, 71–126.
https://doi.org/10.3897/zookeys.549.6889

Nakahara, S. & Monteiro, R.C. (1999) *Frankliniella zucchini* (Thysanoptera: Thripidae), a new species and vector of tospovirus in Brazil. *Proceedings of the Entomological Society of Washington*, 101 (2), 290–294.

Rambaut, A. & Drummond, A.J. (2007) Tracer v1. 4: MCMC trace analyses tool. Available from: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 10 May 2017)

Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System. *Molecular Ecology Notes*, 7, 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rugman-Jones, P.F., Hoddle, M.S. & Stouthamer, R. (2010) Nuclear-Mitochondrial Barcoding Exposes the Global Pest Western Flower Thrips (Thysanoptera: Thripidae) as Two Sympatric Cryptic Species in its native California. *Journal of Economic Entomology*, 103, 877–886. https://doi.org/10.1603/EC09300

ThripsWiki (2017) ThripsWiki—providing information on the World's thrips. Available from: http://thrips.info/wiki/Main_Page (accessed 25 July 2017)